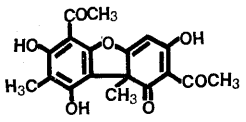
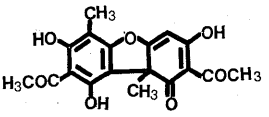
Mariko NUNO*: **On the occurrence of isousnic acid in *Cladonia* species**

布 万里子*: イソウスニン酸のクラドニアに於ける分布について

Recently Shibata and Taguchi[1] isolated (+) isousnic acid from *Cladonia mitis* Sandst. and (−) isousnic acid from *C. pleurota* Schaer. respectively. They considered these isousnic acids together with ordinary usnic acids to be biosynthesized in lichens by the oxydative coupling of two mols of methylphloroacetophenone. Chemical formulae reported by them are as follows:

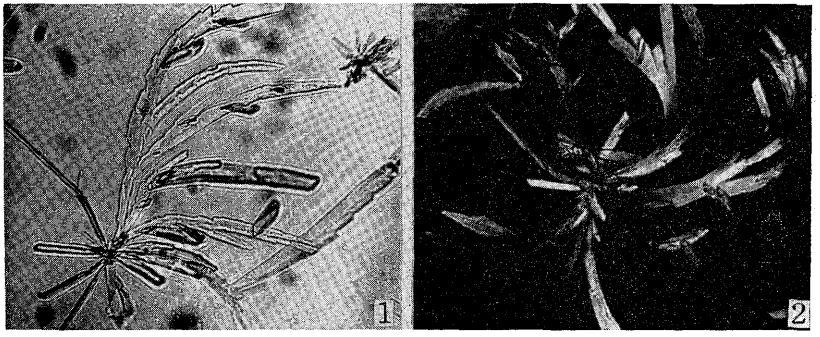


USNIC ACID



ISOUSNIC ACID

Recrystallized from GE solution on glass plate isousnic acid gives, contrary to the straight yellow prisms of ordinary usnic acid, distinctly curved pale yellowish prisms. In the presence of larger amount of ordinary usnic acid the formation of these curved prisms is suppressed and becomes indistinct.



Figs. 1-2. 1. Crystals of isousnic acid recrystallized from GE solution. 2. Crystals of isousnic acid recrystallized from GE solution; with crossed nicols.

* Iatrochemical Research Foundation, 5-35-23, Daita-machi, Setagaya-ku, Tokyo. 財団法人薬理研究会.

1) Tetrahedron Letters, 48, 4867-4871, 1967.

Table 1. Color reaction of usnic and isousnic acids with various reagents.

	usnic acid	isousnic acid
K	yellow	yellowish
KC	deep yellow	yellowish
$FeCl_3$	reddish brown	reddish brown
Ehrlich's reagent	blue	greenish brown

The color reactions of isousnic acid closely resemble those of ordinary one, as shown in Table 1. Therefore, it is impossible to distinguish these acids by the color reaction alone. To make a more definite identification of isousnic and usnic acids thin layer chromatographic tests are indispensable. Cold benzene extract of lichen thalli was spotted on Merck's Silicagel G previously treated with 0.5 N oxalic acid solution and developed by benzene. After the treatment of 10% sulphuric acid, a greenish brown spot at Rf 0.4 represents ordinary usnic acid, whereas a brownish spot at Rf 0.55 is attributed to isousnic acid.

In the course of present study I have tested about one thousand specimens of 57 *Cladonia* species preserved at the National Science Museum, Tokyo. Among them, isousnic acid along with ordinary usnic acid was demonstrated in 8 species; 5 species of *Cladina*, 2 species of *Cocciferae*, and 1 species of *Ochroleucae*, while it was absent in the other 49 species which are listed below. It is noteworthy that isousnic acid is not demonstrated at all in any species of *Unciales*, even though usnic acid is present abundantly. *C. carneola* Fr. has been considered to belong to *Ochrophaeae*, because of the presence of brown apothecia. Among species of *Ochrophaeae*, however, isousnic acid has been demonstrated so far only in *C. carneola*. The occurrence of isousnic acid as well as zeorin seems to suggest that *C. carneola* has a close affinity with *C. pleurota*, though their apothecial discs are of different color.

In addition, isousnic acid was demonstrated by microcrystallization as well as chromatographic test in a species of *Sphaerophorus*[2] collected by Dr. M. Sato in New Zealand. This is the only species which produces isousnic acid but no usnic acid so far as known.

2) It will be described by Dr. M. Sato as a new species.

Species of *Cladonia* tested with chromatographic methods (numerals in parentheses indicate number of specimens tested) are listed below.

(I) Usnic acid and isousnic acid present.

1) *C. arbuscula* (Wallr.) Rabenh. (123)
2) *C. tenuis* (Flk.) Harm. (36) (including *C. tenuiformis* Ahti)
3) *C. mitis* Sandst. (172)
4) *C. submitis* Evans (86)
5) *C. subtenuis* (des Abb.) Evans (21)
6) *C. pleurota* (Flk.) Schaer. (175) (including f. *esorediata* Asahina)
7) *C. deformis* (L.) Hoffm. (27)
8) *C. carneola* (Fr.) Fr. (41)

(II) Usnic acid present but no isousnic acid.

1) *C. alpestris* (L.) Rabenh. (13)
2) *C. impexa* Harm. (3) (including *C. pacifica* Ahti)
3) *C. pseudoevansii* Asahina (5)
4) *C. pycnoclada* (Pers.) Nyl. (1)
5) *C. leptoclada* des Abb. (2)
6) *C.* cf. *alpestroides* des Abb. (2)
7) *C. terrae-novae* Ahti (3)
8) *C. skottsbergii* H. Magn. (1)
9) *C. mediterranea* Duv. & des Abb. (1)
10) *C. pseudomacilenta* Asahina (5)
11) *C. macilenta* Hoffm. ssp. *theiophila* Asah. (3)
12) *C. transcendens* Vain. (5) (including *C. hitatiensis* Asah.)
13) *C. vulcani* Savicz (1)
14) *C. gonecha* (Ach.) Asahina (31)
15) *C. metacorallifera* Asahina (7)
16) *C. incrassata* Flk. (6)
17) *C. heteroclada* Asahina (2)
18) *C. granulans* Vain. (9)
19) *C. yunnana* (Vain.) des Abb. (6)
20) *C. bellidiflora* (Ach.) Schaer. (5)
21) *C. graciliformis* Zahlbr. (5)
22) *C. angustata* Nyl. (5)
23) *C. cristatella* Tuck. (6)
24) *C. abbreviatula* Merrill (1)
25) *C. coccifera* (L.) Willd. (105)
26) *C. leporina* Fr. (8)
27) *C. amaurocraea* (Flk.) Schaer. (6)
28) *C. uncialis* (L.) Web. (7)
29) *C. pseudostellata* Asahina (7)
30) *C. nipponica* Asahina (7)
31) *C. Boryi* Tuck. (5)
32) *C. subsetacea* Robbins. (4)
33) *C. destricta* Nyl. (6)
34) *C. perforata* Evans (1)
35) *C. pachycladodes* Vain. (4)
36) *C. caroliniana* (Schwein.) Tuck. (7)
37) *C. Delavayi* des Abb. (4)
38) *C. medusina* (Bory) Nyl. (4)
39) *C. siamea* des Abb. (1)
40) *C. peltasta* Nyl. (1)
41) *C. botrytes* (Hag.) Willd. (13)

42) *C. koyaensis* Asahina (2)
43) *C. cyanipes* (Sommerf.) Vain. (20)
44) *C. piedomontensis* Merrill (8)
45) *C. bacilliformis* (Nyl.) Vain. (4)
46) *C. robbinsii* Evans (3)
47) *C. endiviaefolia* Fr. (3)
48) *C. retipora* (Labill.) Fr. (7)
49) *C. foliacea* (Huds.) Schaer. (4)

The author is grateful to Dr. Y. Asahina for his guidance and helpful advices and Dr. S. Shibata for his suggestions on chemistry. She is also indebted to Dr. S. Kurokawa for the arrangement of a lot of specimens preserved in the National Science Museum, Tokyo.

* * * *

東大の柴田教授及び田口氏は，地衣のウスニン酸の生合成機構を研究中，日本産の *Cladonia mitis* より，(+) usnic acid の他に其異性体を分離し，之を (+) isousnic acid と命名した。尚其後両氏は，同じく邦産の *Cladonia pleurota* より，(−) usnic acid 及び (−) isousnic acid の分離にも成功している。其構造上からも判明する様に，ウスニン酸の左の核が反転すればイソウスニン酸を生ずるので，地衣体内で二分子の methylphloroacetophenone が酸化的縮合を行って結合する場合，ウスニン酸の異性体としてイソウスニン酸も同時に生ずるものと考えられる。顕微化学的に両者を区別する事は，通常イソウスニン酸と同時に多量に含まれているウスニン酸のために極めて難しいが，薄層クロマトグラフィーではよく分離出来る。筆者は，科学博物館所蔵のウスニン酸を含んでいるクラドニアを，57 種，1050 点について調査した結果，其内，クラヂナ亜属を主とする僅かに 8 種が，多量のウスニン酸と共にイソウスニン酸を含んで居り，残りの 49 種にはイソウスニン酸は含まれていなかった。

尚ついでながら，筆者は偶然にも，佐藤正巳博士採集のニュージーランドの *Sphaerophorus* より，ウスニン酸不含のイソウスニン酸を顕微化学法で，又クロマト法に依り発見した。

地衣成分の生体内に於ける複雑な生合成機構を思う時，イソウスニン酸が単独に，又はウスニン酸と共存して，或はウスニン酸のみが生産されていると云う事実は極めて興味深い。地衣学上，イソウスニン酸を執り上げたのは初めてであるが，今後は分類学的にも甚だ重要な因子となるものと思う。